



RAPID COMMUNICATION

## Intrahepatic HBV DNA as a predictor of antiviral treatment efficacy in HBeAg-positive chronic hepatitis B patients

Hai-Ying Lu, Li-Wei Zhuang, Yan-Yan Yu, Hadad Ivan, Chong-Wen Si, Zheng Zeng, Jun Li, Dong-Ming Hou, Xin-Yue Chen, Zhong-Hou Han, Yong Chen

Hai-Ying Lu, Li-Wei Zhuang, Yan-Yan Yu, Chong-Wen Si, Zheng Zeng, Jun Li, Department of Infectious Diseases, Peking University First Hospital, Beijing 100034, China  
Hadad Ivan, Dong-Ming Hou, Indiana University School of Medicine, Indianapolis, IN 46202, United States  
Xin-Yue Chen, Beijing You'an Hospital, Beijing 100054, China  
Zhong-Hou Han, Qinhuangdao Third Hospital, Qinhuangdao 066000, Hebei Province, China  
Yong Chen, Huai'an Infectious Disease Hospital, Huai'an 223300, Jiangsu Province, China  
Supported by Beijing Municipal Science & Technology Commission, No. H020920020690

Correspondence to: Professor Chong-Wen Si, Department of Infectious Diseases, Peking University First Hospital, Beijing 100034, China. bjsichongwen@sina.com

Telephone: +86-10-66551122-2370

Received: 2006-12-25

Accepted: 2007-01-14

### Abstract

**AIM:** To evaluate the effect of antiviral agents on intrahepatic HBV DNA in HBeAg-positive chronic hepatitis B patients.

**METHODS:** Seventy-one patients received treatment with lamivudine, interferon alpha (IFN- $\alpha$ 2b) or sequential therapy with lamivudine-IFN- $\alpha$ 2b for 48 wk. All subjects were followed up for 24 wk. Serum and intrahepatic HBV DNA were measured quantitatively by PCR. HBV genotypes were analyzed by PCR-RFLP.

**RESULTS:** At the end of treatment, the intrahepatic HBV DNA level in 71 patients decreased from a mean of  $(6.1 \pm 1.0) \log_{10}$  to  $(4.9 \pm 1.4) \log_{10}$ . Further, a larger decrease was seen in the intrahepatic HBV DNA level in patients with HBeAg seroconversion. Intrahepatic HBV DNA level (before and after treatment) was not significantly affected by the patients' HBV genotype, or by the probability of virological flare after treatment.

**CONCLUSION:** Intrahepatic HBV DNA can be effectively lowered by antiviral agents and is a significant marker for monitoring antiviral treatment. Low intrahepatic HBV DNA level may achieve better efficacy of antiviral treatment.

therapy; HBV genotype

Lu HY, Zhuang LW, Yu YY, Ivan H, Si CW, Zeng Z, Li J, Hou DM, Chen XY, Han ZH, Chen Y. Intrahepatic HBV DNA as a predictor of antiviral treatment efficacy in HBeAg-positive chronic hepatitis B patients. *World J Gastroenterol* 2007; 13(20): 2878-2882

<http://www.wjgnet.com/1007-9327/13/2878.asp>

### INTRODUCTION

Hepatitis B virus (HBV) is one of the major causes of liver disease worldwide. Approximately more than 350 million people in the world are chronic carriers, and eventually 15%-25% of them could progress to end-stage liver disease and hepatocellular carcinoma, ranking ninth globally among all causes of mortality (up to 1 million deaths annually)<sup>[1]</sup>. Fortunately, several therapies have been developed for chronic hepatitis B. Anti-viral therapy is believed to be the most important strategy. Currently available anti-viral drugs include interferon-alpha<sup>[2-4]</sup> and nucleoside analogue agents<sup>[5-9]</sup>. It was reported that long-term anti-viral treatment can improve fibrosis and cirrhosis, increase survival rate and decrease the incidence of hepatocellular carcinoma in patients with chronic hepatitis B<sup>[10,11]</sup>. Nonetheless, when the treatment with anti-viral drugs is stopped, relapse occurs in a great majority of patients, even if they have undetectable serum HBV DNA, normal serum alanine aminotransferase level and HBeAg seroconversion.

Therefore, it is important to determine which end points should be used to judge the success or failure of treatment and assist in determining how long therapy should be maintained. Since biopsy specimens from chronic HBV patients are not easy to obtain, intrahepatic HBV DNA level is not often studied. The aim of the present study was to analyze the effect of anti-viral drugs on intrahepatic HBV DNA and the relationship between intrahepatic HBV DNA and the long-term efficacy of antiviral treatment.

### MATERIALS AND METHODS

#### Subjects

Between March 2003 and March 2005, 71 patients (59

Table 1 Histological and virological characteristics of 71 patients with chronic HBV infection mean  $\pm$  SD

Parameter	Total ( <i>n</i> = 71)	HBeAg positive ( <i>n</i> = 54)	HBeAg seroconversion ( <i>n</i> = 17)
Knodel score: pretreatment	8.2 $\pm$ 4.1	8.4 $\pm$ 4.1	7.1 $\pm$ 4.2
post-treatment	5.8 $\pm$ 3.4 <sup>a</sup>	6.0 $\pm$ 3.6	5.1 $\pm$ 3.1 <sup>a</sup>
Intrahepatic HBV DNA (log10): pretreatment	6.1 $\pm$ 1.0	6.3 $\pm$ 0.8	5.6 $\pm$ 1.2
post-treatment	4.9 $\pm$ 1.4 <sup>a</sup>	5.1 $\pm$ 1.5	4.1 $\pm$ 0.8 <sup>a</sup>
Serum HBV DNA (log10): pretreatment	7.7 $\pm$ 1.1	7.9 $\pm$ 0.9	7.5 $\pm$ 1.0
post-treatment	4.0 $\pm$ 1.3 <sup>a</sup>	4.4 $\pm$ 1.4 <sup>a</sup>	3.0 $\pm$ 0.2 <sup>a</sup>
ALT (nkat/L): pretreatment	3390 $\pm$ 2321	3206 $\pm$ 2354	3841 $\pm$ 2371
post-treatment	868 $\pm$ 885 <sup>a</sup>	9853 $\pm$ 985	618 $\pm$ 659 <sup>a</sup>

<sup>a</sup>*P* < 0.05 vs pretreatment.

males and 12 females) with HBeAg-positive chronic hepatitis B were recruited. Their age was 19-47 (mean 32  $\pm$  9) years. Informed consent was obtained from all patients. The patients were matched according to the following criteria: positive for HBsAg and HBeAg, serum HBV DNA  $\geq 1 \times 10^5$  copies/L, serum alanine aminotransferase (ALT) level above at least two-fold the normal range (normal range 0-667 nkat/L) for more than 6 mo. Exclusion criteria were as follows: alcoholism, pregnancy, cirrhosis, chronic renal failure, concurrent autoimmune disease, serious neurological disorders, human immunodeficiency virus (HIV) infection and viral hepatitis A, C, delta or E. Patients treated with interferon or other anti-viral therapies for 6 mo prior to enrollment in this study were also excluded. Patients were treated with 100 mg oral lamivudine daily (*n* = 35), or lamivudine during the first 8 mo and IFN- $\alpha$  from mo 7 to 12 (*n* = 24) or 5 million units of interferon-alpha (IFN- $\alpha$ ), three times per week (*n* = 12). Treatment groups were randomized at the ratio 3:2:1. The total duration of therapy was 12 mo. All subjects were followed up for 24 wk after the 12 mo of treatment. Presence of serum HBV DNA level  $\leq 1 \times 10^3$  and normalization of serum ALT level were assessed as treatment response.

#### Measurement of hepatitis B virus markers and biochemical tests

Blood samples were obtained before and after treatment and at the 24 wk follow-up visit. Liver biochemistry and HBV marker test (enzyme-linked immunosorbant assay) were performed on these samples.

Liver biopsy specimens were collected by needle biopsies (0.5 cm-1.5 cm) before and after treatment. The tissue was washed several times in cold phosphate buffered saline (PBS) and stored at -70°C. Total DNA was extracted from liver tissue with Qiaamp DNA tissue Mini DNA kit (Qiagen, Germany). Serum and intrahepatic HBV DNA were measured quantitatively by real-time polymerase chain reaction (PCR) (Model 5700, ABI Company, USA) with a lower limit of detection of  $1 \times 10^3$  HBV DNA copies/L and  $1 \times 10^3$  copies/g total DNA, respectively. HBV genotypes were determined by PCR restriction fragment length polymorphism (PCR-RFLP) analysis<sup>[12]</sup>.

#### Detection of histological inflammatory score

Histological inflammatory score was detected with the Knodel scoring system. Histological response was defined

as a decrease by at least two points in the Knodel scoring system<sup>[13]</sup>.

#### Statistical analysis

Data were analyzed with the Statistical Program for Social Sciences (SPSS 13.0 for Windows). Categorical variables were tested using chi-square test or Fisher's exact test. Normally distributed variables were tested using *t*-test or ANOVA, whereas continuous variables with skewed distribution were tested using the Kruskal Wallis test. A regression model was used for univariate and multivariate analysis. *P* < 0.05 was considered statistically significant.

## RESULTS

#### Histological, biochemical and virological data

At the end of treatment, the mean values of Knodel score, serum HBV DNA level, intrahepatic HBV DNA level, and serum ALT level in all the 71 patients declined significantly (*P* < 0.05). HBeAg seroconversion occurred in 17 out of the 71 patients. There was no significant difference in above mentioned parameters between HBeAg seroconversion group and HBeAg positive group before treatment (*P* > 0.05), except for the baseline intrahepatic HBV DNA levels (5.6  $\pm$  1.2) log10 and (6.3  $\pm$  0.8) log10, respectively (*P* = 0.02). After treatment, HBeAg seroconversion group had better improvement than HBeAg positive group. The mean intrahepatic HBV DNA decreased to (4.1  $\pm$  0.8) log10 in seroconversion group (*P* = 0.0124), and to (5.1  $\pm$  1.5) log10 (*P* = 0.0872) in HBeAg positive group (Table 1).

#### Intrahepatic HBV DNA level and HBV genotype

At the end of treatment, the intrahepatic HBV DNA load was less than 5 log10 in 38 patients, and higher than 5 log10 in 33 patients (*P* < 0.05). The difference was not statistically significant in serum HBV DNA load, histology and serum ALT level before treatment. After treatment, compared with the patients with intrahepatic HBV DNA load greater than 5 log10, greater reduction in above mentioned parameters was seen in patients with intrahepatic HBV DNA load less than 5 log10. After 24 wk of treatment, sustained virological response rate and ALT normalization rate were very similar between two groups (*P* > 0.05) (Table 2). Regression analysis showed that virological flares after antiviral treatment were not correlated with the baseline or post-treatment level of

Table 2 Intrahepatic HBV DNA level in chronic HBV patients and HBV genotypes mean  $\pm$  SD

Parameter	Intrahepatic HBV DNA < 5 log <sub>10</sub> ( <i>n</i> = 38)	Intrahepatic HBV DNA ≥ 5 log <sub>10</sub> ( <i>n</i> = 33)	HBV genotypes	
			Genotype C group ( <i>n</i> = 61)	Genotype B ( <i>n</i> = 10)
Knodel score: pretreatment	7.6 $\pm$ 4.0	8.2 $\pm$ 4.1	8.1 $\pm$ 4.1	5.7 $\pm$ 3.2
post-treatment	4.8 $\pm$ 2.2 <sup>a</sup>	7.4 $\pm$ 4.1	6.0 $\pm$ 3.4 <sup>a</sup>	5.4 $\pm$ 3.1
Intrahepatic HBV DNA (log <sub>10</sub> ): pretreatment	5.8 $\pm$ 1.0	6.3 $\pm$ 0.9	6.1 $\pm$ 0.9	5.6 $\pm$ 1.5
post-treatment	3.8 $\pm$ 0.9 <sup>a</sup>	6.1 $\pm$ 0.7	4.9 $\pm$ 1.4 <sup>a</sup>	4.7 $\pm$ 1.2
Serum HBV DNA (log <sub>10</sub> ): pretreatment	7.6 $\pm$ 1.0	7.8 $\pm$ 1.3	7.7 $\pm$ 1.2	7.6 $\pm$ 0.8
post-treatment	3.3 $\pm$ 0.7 <sup>a</sup>	4.7 $\pm$ 1.4 <sup>a</sup>	4.05 $\pm$ 1.3 <sup>a</sup>	4.9 $\pm$ 1.2 <sup>a</sup>
Off- treatment 24 wk	5.1 $\pm$ 1.4	5.2 $\pm$ 1.7	5.1 $\pm$ 1.6	4.3 $\pm$ 1.3
ALT (nkat/L): pretreatment	4342 $\pm$ 2788	2571 $\pm$ 1519	3390 $\pm$ 2438	4509 $\pm$ 2338
post-treatment	634 $\pm$ 567 <sup>a</sup>	1135 $\pm$ 1052	912 $\pm$ 918 <sup>a</sup>	551 $\pm$ 236 <sup>a</sup>
Off-treatment 24 wk	1519 $\pm$ 1519	1637 $\pm$ 1369	1503 $\pm$ 1336	1987 $\pm$ 1720
Off-treatment 24 wk virological response rate	23.7% (9/38)	27.3% (9/33)	31.1% (19/51)	30% (3/10)
Off-treatment 24 wk ALT normalization rate	16.7% (15/38)	13.3% (10/33)	39.3% (24/51)	30% (3/10)

<sup>a</sup>*P* < 0.05 vs pretreatment.

intrahepatic HBV DNA load (*P* > 0.05).

HBV genotype C accounted for 85.9% (*n* = 61), and genotype B for 14.1% (*n* = 10). The mean intrahepatic HBV DNA loads in genotype C patients before and after treatment were (6.1  $\pm$  0.9) log<sub>10</sub> and (4.9  $\pm$  1.4) log<sub>10</sub> (*P* < 0.05), and (5.6  $\pm$  1.5) log<sub>10</sub> and (4.7  $\pm$  1.2) log<sub>10</sub> in genotype B patients, respectively (*P* > 0.05). There was no statistically significant difference in serum intrahepatic HBV DNA load and ALT level between the two groups at the end of treatment and in any of the parameters measured after 24 wk of treatment (*P* > 0.05). After 24 wk of treatment, sustained virological response rate and ALT normalization rate were similar (Table 2).

### Antiviral outcome

There was no significant difference in all the parameters among the three groups before treatment (*P* > 0.05). At the end of treatment, the antiviral effect of sequential lamivudine-INF- $\alpha$  therapy and lamivudine monotherapy was similar (*P* > 0.05), which was superior to that of INF- $\alpha$  monotherapy (*P* < 0.05). Reduction of intrahepatic HBV DNA was greater in lamivudine-INF- $\alpha$  therapy group and lamivudine monotherapy group than in INF- $\alpha$  monotherapy group, suggesting that the nucleoside analogue agents might play a stronger role than INF- $\alpha$  in inhibiting intrahepatic HBV DNA (data not shown).

## DISCUSSION

Due to recent advancements, chronic hepatitis B has become a treatable disease. The short-term efficacy of INF- $\alpha$  is about 40%-60%<sup>[12,17]</sup>. Peginterferon-alpha (PEG INF- $\alpha$ ) is more effective against HBeAg-positive chronic hepatitis B than either lamivudine or standard INF- $\alpha$  monotherapy<sup>[3,4]</sup>. Nucleoside analogue agents markedly suppress HBV DNA polymerase, and have numerous advantages, such as minimal side effects, ease of administration. Furthermore, they show good effects on chronic hepatitis B, liver decompensation, cirrhosis, or other coexisting conditions. It was reported that serum HBV DNA is undetectable in 80% of patients, HBeAg is eliminated in 20%-25% of them, and 60% ALT level becomes normal in 60% of them after treatment with

lamivudine<sup>[14-16]</sup>.

Nonetheless, the efficacy of antiviral treatment is far from perfect. INF- $\alpha$  can achieve a sustained response after one year in only 20%-30% of chronic HBV infection patients<sup>[17]</sup>. Relapse occurs in the majority of patients after therapy. The main reason is that HBV covalently closed circular DNA (cccDNA) in infected hepatocytes cannot be eliminated by antiviral agents, leading to rebound of HBV DNA after antiviral therapy<sup>[18]</sup>. The life cycle of HBV relies on a covalently closed circular form of the viral genome, which provides the template for viral pregenomic messenger RNA. Replication of cccDNA is not semi-conservative, and needs viral DNA cycling back to the nuclei to amplify and maintain the pool of cccDNA<sup>[19,20]</sup>. It was reported that intrahepatic HBV cccDNA correlates positively with the total intrahepatic HBV DNA<sup>[21]</sup>, suggesting that intrahepatic HBV DNA should be eliminated first in order to clear HBV cccDNA and intrahepatic HBV DNA level can be taken as a reasonable parameter in evaluating the efficacy of antiviral therapy, thus helping decide the duration of therapy.

The results of our study show that antiviral treatment decreased the serum and intrahepatic HBV DNA as well as alanine aminotransferase levels in 71 patients (*P* < 0.05). HBeAg seroconversion occurred in 17 out of the 71 patients at the end of treatment. There was no significant difference in the baseline levels of Knodel score, serum ALT and HBV DNA levels between HBeAg seroconversion and HBeAg positive groups (*P* > 0.05). Only the intrahepatic HBV DNA level was obviously lower in HBeAg seroconversion group than in HBeAg positive group (*P* = 0.02). After treatment, greater improvement in above mentioned parameters was seen in patients with HBeAg seroconversion than in HBeAg positive patients. The mean intrahepatic HBV DNA level was lower in the HBeAg seroconversion group than in the HBeAg positive group (*P* = 0.010), suggesting that intrahepatic HBV DNA can be effectively lowered by antiviral agents and a lower intrahepatic HBV DNA level is able to predict the efficacy of antiviral therapy.

At the end of treatment, the intrahepatic HBV DNA load was less than 5 log<sub>10</sub> in 38 patients and greater than 5 log<sub>10</sub> in 33 patients. The mean intrahepatic HBV DNA



load before and after treatment was obviously lower in the patients with intrahepatic HBV DNA load less than 5 log<sub>10</sub> than in the patients with intrahepatic HBV DNA load greater than 5 log<sub>10</sub> ( $P < 0.05$ ), and the difference was not statistically significant in the other parameters. At the end of treatment, greater reduction in the measured parameters was seen in patients with intrahepatic HBV DNA load less than 5 log<sub>10</sub>, indicating that reduction in intrahepatic HBV DNA load is also very important in antiviral treatment. After 24 wk of treatment, sustained virus response rate and ALT normalization rate for the two groups were very similar (23.7% and 16.7%, 27.3% and 13.3%, respectively,  $P > 0.05$ ). Regression analysis showed that virus flares after antiviral treatment were not correlated with the baseline or with the post-treatment level of serum and intrahepatic HBV DNA load ( $r < 0.05$ ,  $P > 0.05$ ). A possible reason is that the duration of antiviral treatment in this study was too short to eliminate or largely lessen the intrahepatic HBV DNA load. Therefore, once the antiviral drugs are withdrawn, the majority of patients would experience virus rebound. Intrahepatic HBV DNA was undetectable even if the patients had undetectable serum HBV DNA, indicating that intrahepatic HBV DNA is more useful than serum HBV DNA in monitoring the efficacy of antiviral therapy, and loss of intrahepatic HBV DNA might be crucial for the patients to achieve sustained antiviral response.

HBV is classified into 8 genotypes (A-H), each showing a distinct geographical distribution and disease progression. HBV genotype C is believed to be associated with a higher risk of reactivation and progression to cirrhosis compared to HBV genotype B<sup>[22,23]</sup>. Whether hepatitis B virus (HBV) genotypes influence the response to antiviral treatment remains controversial. It was reported that HBV genotypes do not influence the development of resistance to lamivudine, but influence the severity of liver disease<sup>[24]</sup>. In the present study, 85.9% of patients were infected with the HBV genotype C and 14.1% with the HBV genotype B. There was no significant difference in intrahepatic HBV DNA load, serum ALT and HBV DNA level, as well as in histological findings before and after treatment between the two groups ( $P > 0.05$ ). After 24 wk of treatment, both sustained virological response rate and ALT normalization rate for the two groups were very similar ( $P > 0.05$ ), indicating that HBV genotypes have no influence on intrahepatic HBV DNA load. In this study, there was no statistically significant difference in the long-term effect of antiviral therapy between HBV genotypes B and C.

In conclusion, antiviral therapy can effectively suppress intrahepatic HBV DNA replication. Better efficacy of antiviral treatment can be achieved in patients with a low intrahepatic HBV DNA level. Intrahepatic HBV DNA level at the end of antiviral treatment is correlated with the effect of antiviral therapy, suggesting that loss of intrahepatic HBV DNA may induces less virus flare and can be taken as the optimal endpoint of antiviral treatment. There is no significant difference in intrahepatic HBV DNA level between HBV genotypes C and B.

## ACKNOWLEDGMENTS

The authors thank Xiao-Qi Qin, Department of Medical

Statistics, Peking University First Hospital, for her valuable statistical advice.

## REFERENCES

- 1 **Fact sheets:** Hepatitus B. Geneva: World Health Organization, October 2000
- 2 **Lok AS**, Heathcote EJ, Hoofnagle JH. Management of hepatitis B: 2000--summary of a workshop. *Gastroenterology* 2001; **120**: 1828-1853
- 3 **Lau GK**, Piratvisuth T, Luo KX, Marcellin P, Thongsawat S, Cooksley G, Gane E, Fried MW, Chow WC, Paik SW, Chang WY, Berg T, Flisiak R, McCloud P, Pluck N. Peginterferon Alfa-2a, lamivudine, and the combination for HBeAg-positive chronic hepatitis B. *N Engl J Med* 2005; **352**: 2682-2695
- 4 **Marcellin P**, Lau GK, Bonino F, Farci P, Hadziyannis S, Jin R, Lu ZM, Piratvisuth T, Germanidis G, Yurdaydin C, Diago M, Gurel S, Lai MY, Button P, Pluck N. Peginterferon alfa-2a alone, lamivudine alone, and the two in combination in patients with HBeAg-negative chronic hepatitis B. *N Engl J Med* 2004; **351**: 1206-1217
- 5 **Dienstag JL**, Schiff ER, Wright TL, Perrillo RP, Hann HW, Goodman Z, Crowther L, Condreay LD, Woessner M, Rubin M, Brown NA. Lamivudine as initial treatment for chronic hepatitis B in the United States. *N Engl J Med* 1999; **341**: 1256-1263
- 6 **Marcellin P**, Chang TT, Lim SG, Tong MJ, Sievert W, Shiffman ML, Jeffers L, Goodman Z, Wulfsohn MS, Xiong S, Fry J, Brosgart CL. Adefovir dipivoxil for the treatment of hepatitis B e antigen-positive chronic hepatitis B. *N Engl J Med* 2003; **348**: 808-816
- 7 **Hadziyannis SJ**, Tassopoulos NC, Heathcote EJ, Chang TT, Kitis G, Rizzetto M, Marcellin P, Lim SG, Goodman Z, Ma J, Arterburn S, Xiong S, Currie G, Brosgart CL. Long-term therapy with adefovir dipivoxil for HBeAg-negative chronic hepatitis B. *N Engl J Med* 2005; **352**: 2673-2681
- 8 **Chang TT**, Gish RG, de Man R, Gadano A, Sollano J, Chao YC, Lok AS, Han KH, Goodman Z, Zhu J, Cross A, DeHertogh D, Wilber R, Colonno R, Apelian D. A comparison of entecavir and lamivudine for HBeAg-positive chronic hepatitis B. *N Engl J Med* 2006; **354**: 1001-1010
- 9 **Lai CL**, Shouval D, Lok AS, Chang TT, Cheinquer H, Goodman Z, DeHertogh D, Wilber R, Zink RC, Cross A, Colonno R, Fernandes L. Entecavir versus lamivudine for patients with HBeAg-negative chronic hepatitis B. *N Engl J Med* 2006; **354**: 1011-1020
- 10 **Lok AS**, Lai CL, Leung N, Yao GB, Cui ZY, Schiff ER, Dienstag JL, Heathcote EJ, Little NR, Griffiths DA, Gardner SD, Castiglia M. Long-term safety of lamivudine treatment in patients with chronic hepatitis B. *Gastroenterology* 2003; **125**: 1714-1722
- 11 **Han HL**, Lang ZW. Changes in serum and histology of patients with chronic hepatitis B after interferon alpha-2b treatment. *World J Gastroenterol* 2003; **9**: 117-121
- 12 **Tanaka Y**, Orito E, Yuen MF, Mukaide M, Sugouchi F, Ito K, Ozasa A, Sakamoto T, Kurbanov F, Lai CL, Mizokami M. Two subtypes (subgenotypes) of hepatitis B virus genotype C: A novel subtyping assay based on restriction fragment length polymorphism. *Hepatol Res* 2005; **33**: 216-224
- 13 **Bayraktar Y**, Koseoglu T, Temizer A, Kayhan B, Van Thiel DH, Uzunalioglu B. Relationship between the serum alanine aminotransferase level at the end of interferon treatment and histologic changes in wild-type and precore mutant hepatitis B virus infections. *J Viral Hepat* 1996; **3**: 137-142
- 14 **Dienstag JL**, Perrillo RP, Schiff ER, Bartholomew M, Vicary C, Rubin M. A preliminary trial of lamivudine for chronic hepatitis B infection. *N Engl J Med* 1995; **333**: 1657-1661
- 15 **Lai CL**, Chien RN, Leung NW, Chang TT, Guan R, Tai DI, Ng KY, Wu PC, Dent JC, Barber J, Stephenson SL, Gray DF. A one-year trial of lamivudine for chronic hepatitis B. Asia Hepatitis Lamivudine Study Group. *N Engl J Med* 1998; **339**: 61-68
- 16 **Dienstag JL**, Goldin RD, Heathcote EJ, Hann HW, Woessner M, Stephenson SL, Gardner S, Gray DF, Schiff ER.

- Histological outcome during long-term lamivudine therapy. *Gastroenterology* 2003; **124**: 105-117
- 17 **Wong DK**, Cheung AM, O'Rourke K, Naylor CD, Detsky AS, Heathcote J. Effect of alpha-interferon treatment in patients with hepatitis B e antigen-positive chronic hepatitis B. A meta-analysis. *Ann Intern Med* 1993; **119**: 312-323
- 18 **Nicoll AJ**, Angus PW, Chou ST, Luscombe CA, Smallwood RA, Locarnini SA. Demonstration of duck hepatitis B virus in bile duct epithelial cells: implications for pathogenesis and persistent infection. *Hepatology* 1997; **25**: 463-469
- 19 **Yang W**, Summers J. Integration of hepadnavirus DNA in infected liver: evidence for a linear precursor. *J Virol* 1999; **73**: 9710-9717
- 20 **Zoulim F**. New insight on hepatitis B virus persistence from the study of intrahepatic viral cccDNA. *J Hepatol* 2005; **42**: 302-308
- 21 **Wong DK**, Yuen MF, Yuan H, Sum SS, Hui CK, Hall J, Lai CL. Quantitation of covalently closed circular hepatitis B virus DNA in chronic hepatitis B patients. *Hepatology* 2004; **40**: 727-737
- 22 **Watanabe K**, Takahashi T, Takahashi S, Okoshi S, Ichida T, Aoyagi Y. Comparative study of genotype B and C hepatitis B virus-induced chronic hepatitis in relation to the basic core promoter and precore mutations. *J Gastroenterol Hepatol* 2005; **20**: 441-449
- 23 **Yuen MF**, Sablon E, Tanaka Y, Kato T, Mizokami M, Doutreloigne J, Yuan HJ, Wong DK, Sum SM, Lai CL. Epidemiological study of hepatitis B virus genotypes, core promoter and precore mutations of chronic hepatitis B infection in Hong Kong. *J Hepatol* 2004; **41**: 119-125
- 24 **Kao JH**. Hepatitis B virus genotypes and hepatocellular carcinoma in Taiwan. *Intervirology* 2003; **46**: 400-407

S- Editor Zhu LH L- Editor Wang XL E- Editor Lu W